NCERQA STAR GRANT ABSTRACT

EPA Grant Number: R827442010
Title: Increased Vulnerability of Neonates to Naphthalene and Its Derivatives
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Research Category: Children's Vulnerability to Toxics

Objectives/Hypothesis: Lung disease is the third leading cause of death in the US for adults and *the* leading cause of death in infants under one year of age. Epidemiological studies implicate maternal cigarette smoking as a cause of a number of childhood respiratory diseases, however, the impact of other environmental contaminants on lung disease in infants is not known. Recent studies with laboratory animals have demonstrated that neonates are much more susceptible to pulmonary injury from bioactivated environmental toxicants than are adults. In the lung, the nonciliated bronchiolar epithelial, or Clara cell, is a target of many bioactivated compounds because the mature phenotype contains high levels of P450 monooxygenase activity. Heightened neonatal pulmonary susceptibility occurs even though levels of P450 activity are low in the developing neonatal lung when compared to the high P450 activity of the mature adult lung. The current proposal focuses on the fundamental differences between poorly differentiated target cells in a neonatal lung and the fully differentiated target cells in a mature lung. This proposal will address the following issues: 1) is the increased susceptibility of Clara cells in neonates chemical- and/or species-specific? 2) is the increase in injury in the immature Clara cell due to a difference in the balance of activation and deactivation enzymes in favor of the activation pathways? 3) is the increase in injury to immature Clara cells due to a decreased ability to regulate glutathione pools?

Approach: These studies focus on the simple polyaromatic hydrocarbon (PAH) naphthalene (for which we have already defined age-related differences in the mouse) and compare its acute toxicity in neonates of another species (the rat) whose adults are nonsusceptible to naphthalene. These age-related differences will be compared with the following chemical derivatives of naphthalene: 1-nitronaphthalene, 2-methylnapthalene, and isopropylnaphthalene in both rats and mice. We will examine acute pulmonary cytotoxicity at 6 and 24 hours post-treatment using state-of-the-art techniques to measure epithelial cytotoxicity at both timepoints: confocal imaging of the ability to exclude the nuclear dye ethidium homodimer-1 and high-resolution histopathology. Primary endpoints include differences in the percentage of injured or permeable cells between neonates and adults. Airway level differences in cytotoxicity will also be evaluated because it is well documented that naphthalene and its derivatives produce site-specific injury. We will also compare the bioactivation potential of the differentiating Clara cells

in the neonates and adults by evaluating *in vitro* metabolism. Finally, we will evaluate the ability of the neonates to regulate their glutathione pools in response to the above environmental toxicants using high performance liquid chromatography and quantitative fluorescent imaging.

Expected Results: The results will show that chemical modification of a simple PAH alters acute toxicity in adults and enhances the sensitivity of neonates at doses below the threshold for pulmonary injury in adults. In neonates, the species-differences in cytotoxicity will disappear. We expect that this increase in vulnerability will be due to an imbalance of activating and deactivating enzymes that favors the activation pathway. We also expect that neonates will not regulate their glutathione pools as efficiently as adults, therefore increasing their vulnerability to environmental toxicants.

This study will greatly expand our database of information concerning the potential heightened susceptibility of infants and children to bioactivated pulmonary toxicants. It will also provide a better basis for the extrapolation of data obtained in rodents to the human. This study will aid in identifying various environmental factors which may have the potential to alter the susceptibility of children to toxic substances in the environment.

Supplemental Keywords: chemicals, histology, pathology, biochemistry